

Homology Comparison of Pitilosarcus Gurneyi and Renilla Muleri
GFPs with Aequorea Victoria wt and Enhanced GFPs

Pitilosarcus	MNRNVLKNTGLKEIMSAKASVEGIVNNHVFSMEGFGKGNVLFGNQLMQIR
Renilla	MSKQILKNTCLQEVMSYKVNLEGIVNNHVFTMEGCGKGNILFGNQLVQIR
EGFP (Aequorea)	MSKGEELFTGVVPIL---VELDGDVNGHKFSVSGEGEGDATYGKLTCLKFI
Aequorea	MSKGEELFTGVVPIL---VELDGDVNGHKFSVSGEGEGDATYGKLTCLKFI
	*.: * : : : . : : * * * * * : : * * : : : *
Prim. consensus	MSKGE222TG2V2I2S2KVEL2G2VN2H2FS22GEG2G2A22G222L222
Pitilosarcus	VTKGGPLPFAFDIVSIAFQYGNRTFTKYPDDIA--DYFVQSFPAGFFYER
Renilla	VTKGAPLPFAFDIVSPAFOYGNRTFTKYPNDIS--DYFIQSFPAGFMYER
EGFP	CTTG-KLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMPEGYVQER
Aequorea	CTTG-KLPVPWPTLVTTFSYGVQCFSTRYPDHMKQHDFFKSAMPEGYVQER
	*. * * * . : : : * * : * : * : : : * : * . : : * * . *
Prim. cons.	2T2G22LP2222222T2FQYG222F22YPD22KQHD2FK222P2G2V2ER
	Chromophore
Pitilosarcus	NLRFEDGAIVDIRSDISLEDDKFHYKVEYRGNGFPSNGPVMQKAILGMEP
Renilla	TLRYEDGGLVEIRSDINLIEDKFVYRVEYKGSNFPDDGPVMQKTIILGIEP
EGFP	TIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYN
Aequorea	TIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYN
	. : : * . . . * : : : * . : : * : * . * . : : : *
Prim. cons.	T22F2D2GN2K2R222K2EGD22V2R2E2KGIDF2EDG22222K222N22
Pitilosarcus	SFEVVMYMN----SGVLVGEVDLVYKLESGNYYSCHMKTfYRSKGGVKEFP
Renilla	SFEAMYMN----NGVLVGEVILVYKLNSGKYYSCHMKTLMKSKGVVKEFP
EGFP	SHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLP
Aequorea	SHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLP
	* . . * . * : * . : . . : * : . : *
Prim. cons.	S22VY2M2DKQKNG22V222I22222D22V2222H222NTP222G2222P
Pitilosarcus	EYHFIHHRLEKTYVEEGSFVEQHETAIAQLTTIGKPLGSLHEWV
Renilla	SYHFIQHRLEKTYVEDGGFVEQHETAIAQMTSIGKPLGSLHEWV
EGFP	DNHYLS--TQSALSKDPNEKRDHMLLEFVTAAGITLGMDELYK
Aequorea	DNHYLS--TQSALSKDPNEKRDHMLLEFVTAAGITLGMDELYK
	. * : : : : : : : * . : : * * . * . :
Prim. cons.	D2H22SHR222222D2N2222H22222VTA2G22LG222222

Alignment data :

Pitilosarcus G. vs. Aequorea

Identity (*) : 55 is 22.45 %
Strongly similar (:) : 61 is 24.90 %
Weakly similar (.) : 35 is 14.29 %
Different : 94 is 38.37 %\

Renilla M. Vs. Aequorea

Identity (*) : 60 is 24.39 %
Strongly similar (:) : 65 is 26.42 %
Weakly similar (.) : 28 is 11.38 %
Different : 93 is 37.80 %

Renilla vs. Pitilosarcus

Alignment length : 238
Identity (*) : 184 is 77.31 %
Strongly similar (:) : 31 is 13.03 %
Weakly similar (.) : 14 is 5.88 %
Different : 9 is 3.78 %

Beau Peelle 10/15/99

Figure 1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GGTTATACAC	AAGTGTATCG	CGTATCTGCA	GACGCATCTA	GTGGGATTAT	TCGAGCGGTA	60
GTATTTACGT	CAGACCTGTC	TAATCGAAAC	CACAACAAAC	TCTTAAATA	AGCCACATTT	120
ACATAATATC	TAAGAGACGC	CTCATTTAAG	AGTAGTAAAA	ATATAATATA	TGATAGAGTA	180
TACAACCTCTC	GCCTTAGACA	GACAGTGTGC	AACAGAGTAA	CTCTTGTTAA	TGCAATCGAA	240
AGCGTCAAGA	GAGATAAG	ATG AGT AAA	CAA ATA TTG	AAG AAC ACT	TGT TTA	291
	Met Ser Lys	Gln Ile Leu	Lys Asn Thr	Cys Leu		
	1	5		10		
CAA GAA GTA	ATG TCG TAT	AAA GTA AAT	CTG GAA GGA	ATT GTA AAC	AAC	339
Gln Glu Val	Met Ser Tyr	Lys Val Asn	Leu Glu Gly	Ile Val Asn	Asn	
	15	20		25		
CAT GTT TTT	ACA ATG GAG	GGT TGC GGC	AAA GGG AAT	ATT TTA TTC	GGC	387
His Val Phe	Thr Met Glu	Gly Cys Gly	Lys Gly Asn	Ile Leu Phe	Gly	
	30	35		40		
AAT CAA CTG	GTT CAG ATT	CGT GTC ACG	AAA GGG GCC	CCA CTG CCT	TTT	435
Asn Gln Leu	Val Gln Ile	Arg Val Thr	Lys Gly Ala	Pro Leu Pro	Phe	
	45	50		55		
GCA TTT GAT	ATT GTG TCA	CCA GCT TTT	CAA TAT GGC	AAC CGT ACT	TTC	483
Ala Phe Asp	Ile Val Ser	Pro Ala Phe	Gln Tyr Gly	Asn Arg Thr	Phe	
	60	65		70		
ACG AAA TAT	CCG AAT GAT	ATA TCA GAT	TAT TTT ATA	CAA TCA TTT	CCA	531
Thr Lys Tyr	Pro Asn Asp	Ile Ser Asp	Tyr Phe Ile	Gln Ser Phe	Pro	
	80	85		90		
GCA GGA TTT	ATG TAT GAA	CGA ACA TTA	CGT TAC GAA	GAT GGC GGA	CTT	579
Ala Gly Phe	Met Tyr Glu	Arg Thr Leu	Arg Tyr Glu	Asp Gly Gly	Leu	
	95	100		105		
GTT GAA ATT	CGT TCA GAT	ATA AAT TTA	ATA GAA GAC	AAG TTC GTC	TAC	627
Val Glu Ile	Arg Ser Asp	Ile Asn Leu	Ile Glu Asp	Lys Phe Val	Tyr	
	110	115	120			
AGA GTG GAA	TAC AAA GGT	AGT AAC TTC	CCA GAT GAT	GGT CCC GTC	ATG	675
Arg Val Glu	Tyr Lys Gly	Ser Asn Phe	Pro Asp Asp	Gly Pro Val	Met	
	125	130		135		
CAG AAG ACT	ATC TTA GGA	ATA GAG CCT	TCA TTT GAA	GCC ATG TAC	ATG	723
Gln Lys Thr	Ile Leu Gly	Ile Glu Pro	Ser Phe Glu	Ala Met Tyr	Met	
	140	145		150		
AAT AAT GGC	GTC TTG GTC	GGC GAA GTA	ATT CTT GTC	TAT AAA CTA	AAC	771
Asn Asn Gly	Val Leu Val	Gly Glu Val	Ile Leu Val	Tyr Lys Leu	Asn	
	160		165		170	
TCT GGG AAA	TAT TAT TCA	TGT CAC ATG	AAA ACA TTA	ATG AAG TCG	AAA	819
Ser Gly Lys	Tyr Tyr Ser	Cys His Met	Lys Thr Leu	Met Lys Ser	Lys	
	175	180		185		
GGT GTA GTA	AAG GAG TTT	CCT TCG TAT	CAT TTT ATT	CAA CAT CGT	TTG	867
Gly Val Val	Lys Glu Phe	Pro Ser Tyr	His Phe Ile	Gln His Arg	Leu	
	190	195		200		
GAA AAG ACT	TAC GTA GAA	GAC GGG GGG	TTC GTT GAA	CAG CAT GAG	ACT	915
Glu Lys Thr	Tyr Val Glu	Asp Gly Gly	Phe Val Glu	Gln His Glu	Thr	
	205	210		215		
GCT ATT GCT	CAA ATG ACA	TCT ATA GGA	AAA CCA CTA	GGA TCC TTA	CAC	963
Ala Ile Ala	Gln Met Thr	Ser Ile Gly	Lys Pro Leu	Gly Ser Leu	His	
	220	225	230		235	
GAA TGG GTT	TAA ACACAGTTAC	ATTACTTTTTT	CCAATTCGTG	TTTCATGTCA	AATAAT	1021
Glu Trp Val	*					
AATTTTTTAA	ACAATTATCA	ATGTTTTGTG	ATATGTTTGT	AAAAAAAAAA	AAAAAAAA	1079

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

TCGGCACGAG	CTGGCCTCCA	CACTTTAGAC	AAA	ATG	AAC	CGC	AAC	GTA	TTA	AAG	54					
				Met	Asn	Arg	Asn	Val	Leu	Lys						
				1				5								
AAC	ACT	GGA	CTG	AAA	GAG	ATT	ATG	TCG	GCA	AAA	GCT	AGC	GTT	GAA	GGA	102
Asn	Thr	Gly	Leu	Lys	Glu	Ile	Met	Ser	Ala	Lys	Ala	Ser	Val	Glu	Gly	
		10					15					20				
ATC	GTG	AAC	AAT	CAC	GTT	TTT	TCC	ATG	GAA	GGA	TTT	GGA	AAA	GGC	AAT	150
Ile	Val	Asn	Asn	His	Val	Phe	Ser	Met	Glu	Gly	Phe	Gly	Lys	Gly	Asn	
		25				30					35					
GTA	TTA	TTT	GGA	AAC	CAA	TTG	ATG	CAA	ATC	CGG	GTT	ACA	AAG	GGA	GGT	198
Val	Leu	Phe	Gly	Asn	Gln	Leu	Met	Gln	Ile	Arg	Val	Thr	Lys	Gly	Gly	
		40			45					50					55	
CCG	TTG	CCA	TTC	GCT	TTC	GAT	ATT	GTT	TCC	ATA	GCT	TTC	CAA	TAC	GGG	246
Pro	Leu	Pro	Phe	Ala	Phe	Asp	Ile	Val	Ser	Ile	Ala	Phe	Gln	Tyr	Gly	
				60					65					70		
AAT	CGC	ACT	TTC	ACG	AAA	TAC	CCA	GAC	GAC	ATT	GCG	GAC	TAC	TTT	GTT	294
Asn	Arg	Thr	Phe	Thr	Lys	Tyr	Pro	Asp	Asp	Ile	Ala	Asp	Tyr	Phe	Val	
			75					80					85			
CAA	TCA	TTC	CCG	GCT	GGA	TTT	TTC	TAC	GAA	AGA	AAT	CTA	CGC	TTT	GAA	342
Gln	Ser	Phe	Pro	Ala	Gly	Phe	Phe	Tyr	Glu	Arg	Asn	Leu	Arg	Phe	Glu	
		90					95					100				
GAT	GGC	GCC	ATT	GTT	GAC	ATT	CGT	TCA	GAT	ATA	AGT	TTA	GAA	GAT	GAT	390
Asp	Gly	Ala	Ile	Val	Asp	Ile	Arg	Ser	Asp	Ile	Ser	Leu	Glu	Asp	Asp	
	105					110					115					
AAG	TTC	CAC	TAC	AAA	GTG	GAG	TAT	AGA	GGC	AAC	GGT	TTC	CCT	AGT	AAC	438
Lys	Phe	His	Tyr	Lys	Val	Glu	Tyr	Arg	Gly	Asn	Gly	Phe	Pro	Ser	Asn	
	120				125					130					135	
GGA	CCC	GTG	ATG	CAA	AAA	GCC	ATC	CTC	GGC	ATG	GAG	CCA	TCG	TTT	GAG	486
Gly	Pro	Val	Met	Gln	Lys	Ala	Ile	Leu	Gly	Met	Glu	Pro	Ser	Phe	Glu	
				140					145					150		
GTG	GTC	TAC	ATG	AAC	AGC	GGC	GTT	CTG	GTG	GGC	GAA	GTA	GAT	CTC	GTT	534
Val	Val	Tyr	Met	Asn	Ser	Gly	Val	Leu	Val	Gly	Glu	Val	Asp	Leu	Val	
			155					160					165			
TAC	AAA	CTC	GAG	TCA	GGG	AAC	TAT	TAC	TCG	TGC	CAC	ATG	AAA	ACG	TTT	582
Tyr	Lys	Leu	Glu	Ser	Gly	Asn	Tyr	Tyr	Ser	Cys	His	Met	Lys	Thr	Phe	
		170					175					180				
TAC	AGA	TCC	AAA	GGT	GGA	GTG	AAA	GAA	TTC	CCG	GAA	TAT	CAC	TTT	ATC	630
Tyr	Arg	Ser	Lys	Gly	Gly	Val	Lys	Glu	Phe	Pro	Glu	Tyr	His	Phe	Ile	
	185					190					195					

Figure 3A
(cont.)

[illegible]

(cont.) Figure 3A

Figure 3B (cont.)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

															GACAAA	ATG	AAC	CGC	AAC	GTA	TTA	AAG	27
															Met	Asn	Arg	Asn	Val	Leu	Lys		
															1				5				
AAC	ACT	GGA	CTG	AAA	GAG	ATT	ATG	TCG	GCA	AAA	GCT	AGC	GTT	GAA	GGA	75							
Asn	Thr	Gly	Leu	Lys	Glu	Ile	Met	Ser	Ala	Lys	Ala	Ser	Val	Glu	Gly								
															10				20				
ATC	GTG	AAC	AAT	CAC	GTT	TTT	TCC	ATG	GAA	GGA	TTT	GGA	AAA	GGC	AAT	123							
Ile	Val	Asn	Asn	His	Val	Phe	Ser	Met	Glu	Gly	Phe	Gly	Lys	Gly	Asn								
															25				35				
GTA	TTA	TTT	GGA	AAC	CAA	TTG	ATG	CAA	ATC	CGG	GTT	ACA	AAG	GGA	GGT	171							
Val	Leu	Phe	Gly	Asn	Gln	Leu	Met	Gln	Ile	Arg	Val	Thr	Lys	Gly	Gly								
															40				50		55		
CCG	TTG	CCA	TTC	GCT	TTC	GAC	ATT	GTT	TCC	ATA	GCT	TTC	CAA	TAC	GGG	219							

Pro	Leu	Pro	Phe	Ala 60	Phe	Asp	Ile	Val	Ser 65	Ile	Ala	Phe	Gln	Tyr 70	Gly		
AAT Asn	CGC Arg	ACT Thr	TTC Phe 75	ACG Thr	AAA Lys	TAC Tyr	CCA Pro	GAC Asp 80	GAC Asp	ATT Ile	GCG Ala	GAC Asp	TAC Tyr 85	TTT Phe	GTT Val	267	
CAA Gln	TCA Ser	TTT Phe 90	CCG Pro	GCT Ala	GGA Gly	TTT Phe	TTC Phe 95	TAC Tyr	GAA Glu	AGA Arg	AAT Asn	CTA Leu 100	CGC Arg	TTT Phe	GAA Glu	315	
GAT Asp	GGC Gly 105	GCC Ala	ATT Ile	GTT Val	GAC Asp	ATT Ile 110	CGT Arg	TCA Ser	GAT Asp	ATA Ile	AGT Ser 115	TTA Leu	GAA Glu	GAT Asp	GAT Asp	363	
AAG Lys 120	TTC Phe	CAC His	TAC Tyr	AAA Lys	GTG Val 125	GAG Glu	TAT Tyr	AGA Arg	GGC Gly	AAC Asn 130	GGT Gly	TTC Phe	CCT Pro	AGT Ser	AAC Asn 135	411	
GGA Gly	CCC Pro	GTG Val	ATG Met	CAA Gln 140	AAA Lys	GCC Ala	ATC Ile	CTC Leu	GGC Gly 145	ATG Met	GAG Glu	CCA Pro	TCG Ser	TTT Phe 150	GAG Glu	459	
GTG Val	GTC Val	TAC Tyr	ATG Met 155	AAC Asn	AGC Ser	GGC Gly	GTT Val	CTG Leu 160	GTG Val	GGC Gly	GAA Glu	GTA Val	GAT Asp 165	CTC Leu	GTT Val	507	
TAC Tyr	AAA Lys	CTC Leu 170	GAG Glu	TCA Ser	GGG Gly	AAC Asn	TAT Tyr 175	TAC Tyr	TCG Ser	TGC Cys	CAC His	ATG Met 180	AAA Lys	ACG Thr	TTT Phe	555	
TAC Tyr	AGA Arg 185	TCC Ser	AAA Lys	GGT Gly	GGA Gly	GTG Val 190	AAA Lys	GAA Glu	TTC Phe	CCG Pro	GAA Glu 195	TAT Tyr	CAC His	TTT Phe	ATC Ile	603	
CAT His 200	CAT His	CGT Arg	CTG Leu	GAG Glu	AAA Lys 205	ACC Thr	TAC Tyr	GTG Val	GAA Glu	GAA Glu 210	GGA Gly	AGC Ser	TTC Phe	GTG Val	GAA Glu 215	651	
CAA Gln	CAC His	GAG Glu	ACG Thr 220	GCC Ala	ATT Ile	GCA Ala	CAA Gln	CTG Leu 225	ACC Thr 225	ACA Thr	ATT Ile	GGA Gly	AAA Lys 230	CCT Pro	CTG Leu	699	
GGC Gly	TCC Ser	CTT Leu	CAT His	GAA Glu	TGG Trp	GTG Val	TAG	AAAATGACCA			ATATACTGGG		GAAAATCACC			753	
AATATACTGG			GGAAAATGAC			CAATTTACTG			GGGAAAATGA			CCAATATACT			GTAGAAAATC		813
ACCAATATAC			TGGGGGAAAT			GACCAATTTA			CTGGGGGAAAT			GACCAATTTA			CTGTAGAAAA		873
TCACCAATAT			ACTGTGGAAA			ATGACCAAAA			TACTGTAGAA			ATGTTACAC			TGGGTTGATA		933
ACCGTTTCGA			TAACCGTTTG			GAAGCTTGTTG			TATACAAGTT			ATTTGGGGTC			ATTTTGTAAAT		993
GTGTATGTGT			GTTGTATGAT			CTATAGACGT			CGTCATTCAT			AGCTTGAATC			CTTCAGCAAA		1053
AGAAACCTCG			AAGCATATTG			AAACCTCGAC			GGAGAGCATA			AAGAGACCGC			ACGTACACAA		1113
ATTATAATAC			CAGCAGTTGG			AATCTTTTAA			CCGATCAAAA			CTATTAAATAT			ATATATACAC		1173
CCTGTATAAC			ATATATATAT			ATATATATCT			ACATAGTTTG			ATATTGATTA			AATCTGTTCT		1233
TGATCACTAA			AAAAAAAAAA			AAAAAAAAAA			AAAAAAAAAA								

(cont.) Figure 3B